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*Aquatic Plant Control Research Program*

# Potential of a Pathogen, *Mycoleptodiscus terrestris*, as a Biocontrol Agent for the Management of *Myriophyllum spicatum* in Lake Guntersville Reservoir

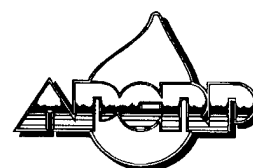
by Judy F. Shearer

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by Judy F. Shearer

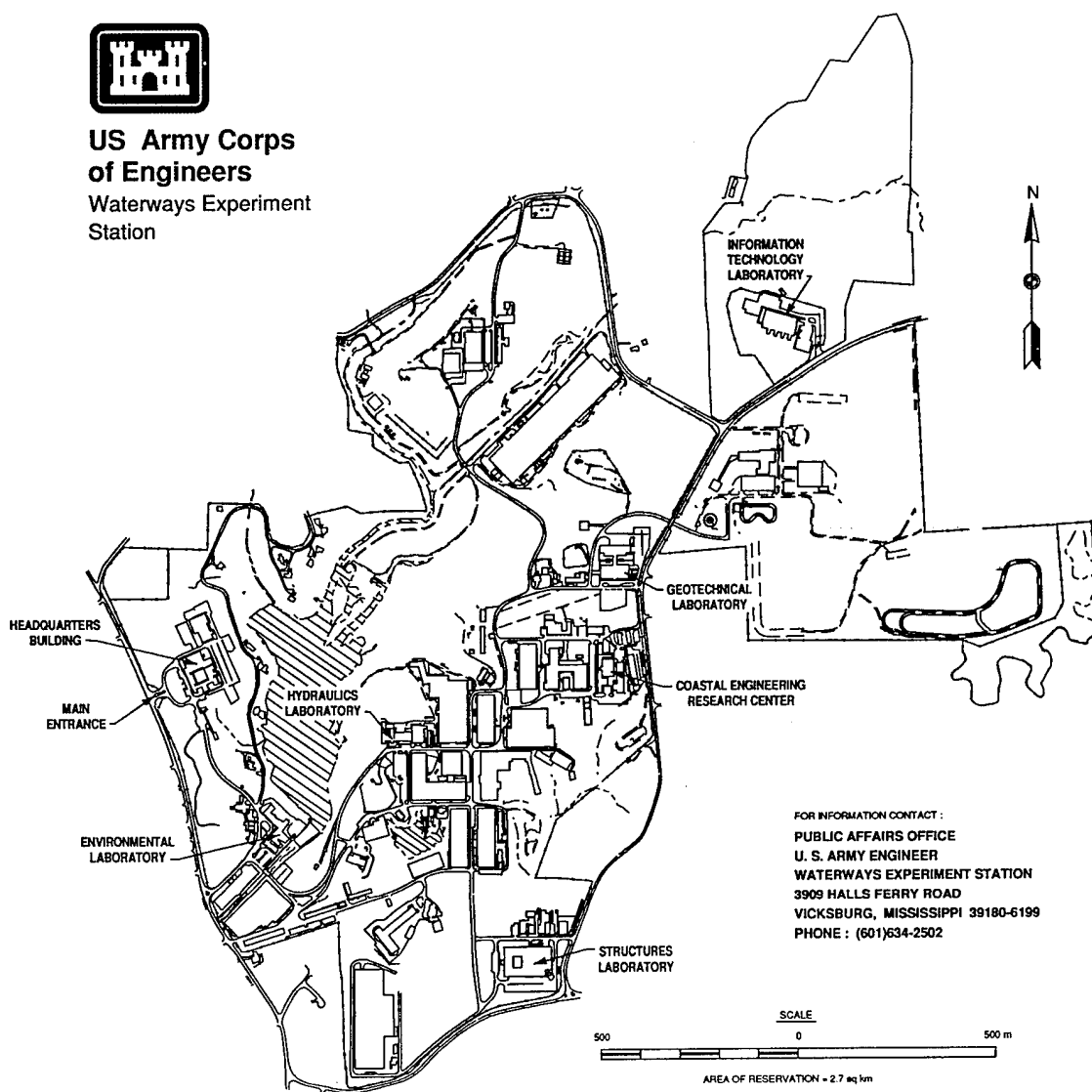
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Final report

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# Preface

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The work reported herein was conducted as part of the Aquatic Plant Control Research Program (APCRP), Work Unit 32200. The APCRP is sponsored by the Headquarters, U.S. Army Corps of Engineers (HQUSACE), and is assigned to the U.S. Army Engineer Waterways Experiment Station (WES) under the purview of the Environmental Laboratory (EL). Funding was provided under Department of the Army Appropriation No. 96X3122, Construction General. The APCRP is managed under the Environmental Resources Research and Assistance Programs (ERRAP), Mr. J. L. Decell, Manager. Mr. Robert C. Gunkel, Jr., was Assistant Manager, ERRAP, for the APCRP. Program Monitor during this study was Ms. Denise White, HQUSACE.

The work reported herein was conducted by scientists and technicians from the EL of WES and from EcoScience Corporation, Worcester, MA. The report was written by Dr. Judy F. Shearer, Aquatic Ecology Branch (AEB), Ecological Research Division (ERD), EL. Mr. Douglas Murphy and Mr. David Brewster of the Guntersville Reservoir Aquatic Research Facility, Tennessee Valley Authority, assisted in the field work. Dr. Michael Grodowitz and Mr. Harvey Jones of EL reviewed the report. The author would like to thank Ms. Janis Lanier, WES, for her technical assistance. The cooperation of EcoScience for providing Aqua-Fyte is also appreciated.

This work was conducted under the general supervision of Dr. Alfred F. Cofrancesco, Chief, AEB, Dr. Conrad J. Kirby, Chief, ERD, and Dr. John W. Keeley, Director, EL.

At the time of publication of this report, Director of WES was Dr. Robert W. Whalin. Commander was COL Bruce K. Howard, EN.

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# 1 Introduction

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*Myriophyllum spicatum* L. (Eurasian watermilfoil), one of the most troublesome aquatic macrophytes, infests freshwater lakes throughout North America (Smith and Barko 1990). Following an intentional planting at Watts Bar Reservoir in the 1960s, it spread throughout the Tennessee Valley Authority (TVA) reservoir system in subsequent years (TVA 1987). Milfoil became particularly problematic at Guntersville Reservoir located in Jackson and Marshall counties of Alabama and in Marion County of Tennessee in the 1980s, where in association with other aquatic macrophytes much of the total surface area of the reservoir was covered (Burns, Bates, and Webb 1990). In locations where conditions are optimum for its growth, milfoil impedes navigation and interferes with flood control, hydroelectric production, and recreational activities.

In the management program developed for Guntersville Reservoir, an array of strategies using chemical, biological, environmental, and mechanical means were proposed for the purpose of controlling nuisance populations of submersed aquatic plants including milfoil (TVA and U.S. Army Corps of Engineers 1990). Historically, chemical control was the method most commonly implemented by TVA for management of aquatic macrophyte populations. With increasing public pressure and concern over herbicide use, a reevaluation of chemical effects and searches for alternative strategies for macrophyte management became important.

Mechanical control methods proposed were of two types: harvester machines designed to cut aquatic vegetation and benthic barriers that reduce growth of vegetation. Environmental methods focused primarily on manipulation of water levels, which in turn alter the environment of aquatic plant populations exposing them to drying conditions. Herbicide effects in Guntersville Reservoir were evaluated by a monitoring research effort that evaluated herbicide residues in water, plants, sediments, and animals, herbicide dissipation rates following applications, and herbicide effects on fish and mollusks (Rodgers, Dunn, and Robison 1992).

Suggested biological management alternatives included the use of fish, insects, and microbial pathogens. At the start of the project, no host specific insects were available for milfoil management; but an ephydrid fly, *Hydrillia paskistanae*, was proposed for hydrilla management. The grass carp,

*Ctenopharyngodon idella* Val. (white amur), was introduced for submersed macrophyte management in 1990. During April-June, 100,000 grass carp were released in Lake Guntersville Reservoir. The fish, while effective, has severe drawbacks that prohibit it from being a good biocontrol agent. First of all, it is highly selective in its feeding, thereby impacting the native (desirable) as well as the exotic plant species. In addition, milfoil is one of the grass carp's least preferred plant species. Inhibitory costs of mechanical removal, increasing pressures to reduce herbicide use, and the unavailability of milfoil-feeding insects made the search for host-specific biological organisms for milfoil management an important research effort.

The use of classical biocontrol techniques for milfoil management was not a viable option when the Joint Agency Guntersville Project sponsored by the TVA and Headquarters, U.S. Army Corps of Engineers began. No host specific insects had been approved for release, and overseas searches for pathogens had not yet been initiated. A microbial biocontrol strategy, the inundative method, utilizing an endemic plant pathogen known to be a weak parasite on milfoil was proposed. Applied at a high dosage rate, a microbial pathogen has the potential to create a disease epidemic in a susceptible plant population resulting in weed control similar to that achieved by conventional herbicides. Successful development of fungi as inundative agents has been documented by the registration of three products in the United States and one in Canada (Harris 1993).

A cellulolytic fungus, *Mycocleptodiscus terrestris* (Gerd.) Ostazeski (Mt), was isolated from diseased milfoil in Massachusetts in the late 1970s and had been demonstrated to have promise as an inundative biocontrol agent for milfoil in laboratory and greenhouse trials (Gunner 1983, 1985; Gunner et al. 1988). A successful field trial was conducted on a milfoil-infested pond (Stockbridge Bowl) in Stockbridge, MA (Gunner 1987). A mycelial suspension of Mt applied in two successive applications to a 15-m<sup>2</sup> plot resulted in 16-fold reductions in stem-leaf biomass compared with an untreated control. Effective application rates (i.e., allowing for the dilution factor) for the two treatments were estimated to be 10 and 38 colony-forming units (CFUs)/milliliter, respectively.

Following the success of laboratory and field trials, a formulation in which mycelium of the fungus was incorporated into a calcium alginate matrix was developed by EcoScience, Inc., Worcester, MA, and registered as the myco-herbicide Aqua-Fyte. The formulation was designed to deliver the active ingredient in a two-step process. Hyphae of the pathogen growing out of the formulation medium provided primary inoculum for infection of stems and leaves. Secondary infection cycles would be initiated by sporulation of the fungus on the surface of the formulated matrix and from disease lesions on the stems and leaves of the infected plant.

Introduction of microbes for biological control purposes against nuisance pest populations is strictly regulated by individual State agencies and two Federal agencies, including the U.S. Environmental Protection Agency (EPA)

and the Agriculture Protection Health Inspection Service (APHIS). The use of a pathogen that is known to be endemic within a State lessens the fear associated with introducing a microbe into a new area. Documentation that Mt occurred in Alabama would greatly facilitate the permitting process from State and Federal agencies. While substantial information including geographic distribution is available on pathogens of terrestrial plants, such information is almost totally lacking for submersed aquatic plants.

The objectives of the study were to (a) survey milfoil populations at Guntersville Reservoir for Mt and other plant pathogens, (b) evaluate the mycoherbicide Aqua-Fyte for control of milfoil, and (c) evaluate the pathogen Mt for biocontrol efficacy on milfoil.

## 2 Materials and Methods

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### Pathogen Survey

Ten plant samples were collected from each of three milfoil-infested areas on Lake Guntersville Reservoir, July 1991 (Figure 1) for microbial analysis. Areas of North Sauty Creek, Mud Creek, and Comer Bridge were chosen for sampling because extensive populations of milfoil still existed in those areas even though it had declined significantly in much of the reservoir since 1988. Milfoil was collected using a garden rake. Plants were examined in situ and diseased leaf and stem tissue placed in sterile plastic bags, kept cool in an insulated chest, and transported to the biocontrol laboratory at the U.S. Army Engineer Waterways Experiment Station (WES) in Vicksburg, MS, for processing.

Plant samples were washed thoroughly with tap water to rid them of surface debris. Each sample was separated into distinct stems and carefully examined using a stereoscopic microscope at  $6\times$  magnification. From each sample, 15 diseased stem and/or leaf sections approximately 1 cm in length were excised and placed in sterile water. The pieces were transferred to a 1.5-percent hypochlorite solution for 1 min to ensure surface sterilization and subsequently rinsed in sterile water. Three stem pieces were placed between slits cut into Martin's agar ( $\text{H}_2\text{O}$ , 1 l; agar, 17 g;  $\text{KH}_2\text{PO}_4$ , 0.5 g;  $\text{K}_2\text{HPO}_4$ , 0.5 g;  $\text{MgSO}_4 \cdot 7 \text{H}_2\text{O}$ , 0.5 g; peptone, 0.5 g; dextrose, 10 g; yeast extract, 0.5 g; rose bengal, 0.05 g; streptomycin, 0.03 g) plates and placed in a dark chamber set at  $28^\circ\text{C}$  for 4 days. Fungal colonies growing out from the stem pieces were counted, picked onto potato dextrose agar (PDA) (DIFCO Laboratories, Detroit, MI) slants, and allowed to grow at room temperature (approximately  $24^\circ\text{C}$ ) for 7 to 10 days. When colonies became well established in the culture tubes, they were placed in a refrigeration unit at  $4^\circ\text{C}$  for temporary storage until they could be identified and accessioned into a permanent collection housed in a cryofreezer.

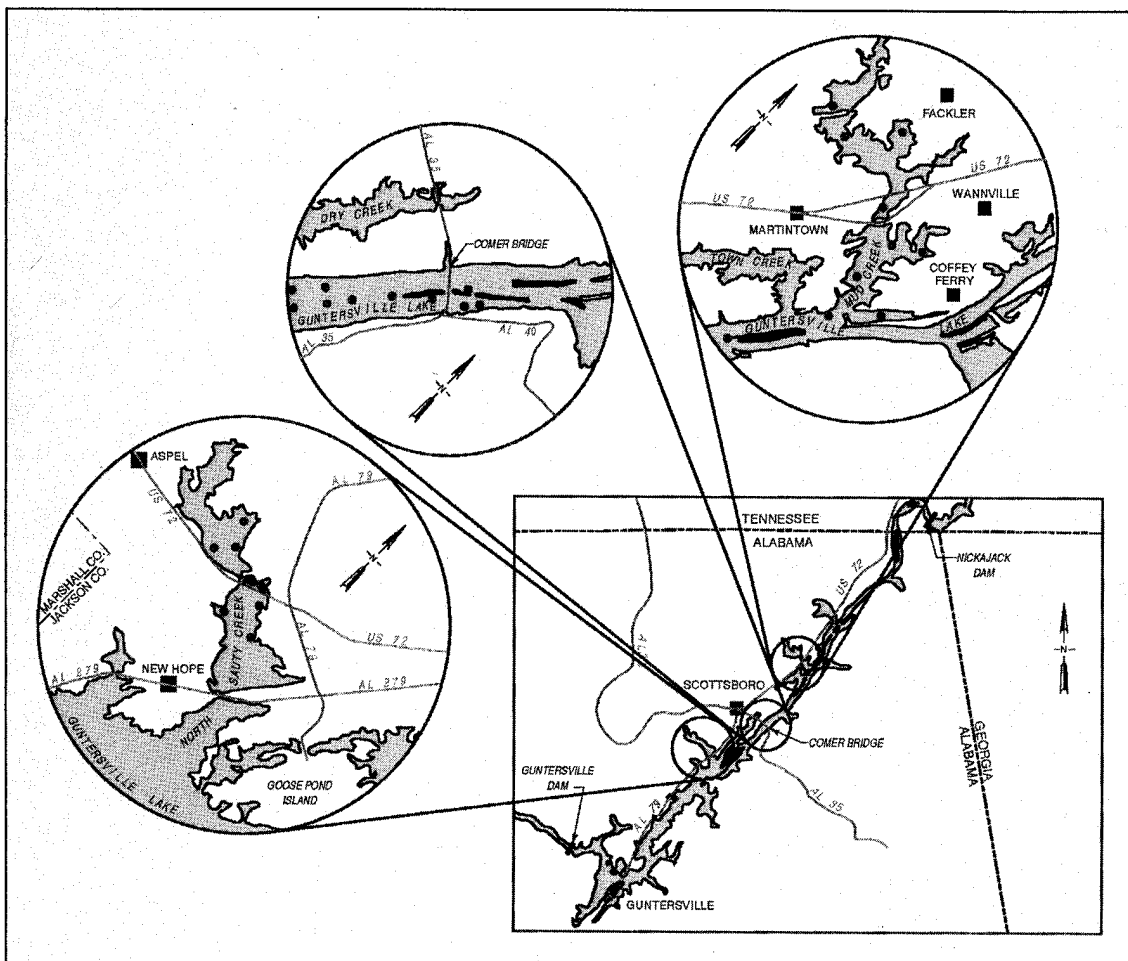


Figure 1. Map of Guntersville Reservoir in Alabama showing collecting sites at North Sauty Creek, Comer Bridge, and Mud Creek

## Aqua-Fyte Evaluation

The mycoherbicide Aqua-Fyte was provided by EcoScience, Inc., Worcester, MA. The active ingredient, *M. terrestris*, was grown in large industrial fermentors, incorporated into a biodegradable medium of calcium alginate, and exuded as 20-percent strings. For field testing, the strings were cut into pieces approximately 2 by 20 mm (Figure 2).

Field plots were set up on a 25-ha milfoil-infested pond located at the Murphy Hill Guntersville Reservoir Aquatic Research Facility adjacent to Guntersville Reservoir (Figure 3). Four paired plots 10 by 10 by 1.5 m separated by 100-m buffer zones were set up in dense stands of milfoil vegetation. Treatments consisted of one application rate of the mycoherbicide at 70 lb (dry wt) active ingredient (ai)/acre paired with untreated controls. Treatments were randomly assigned to the paired plots. To prevent cross

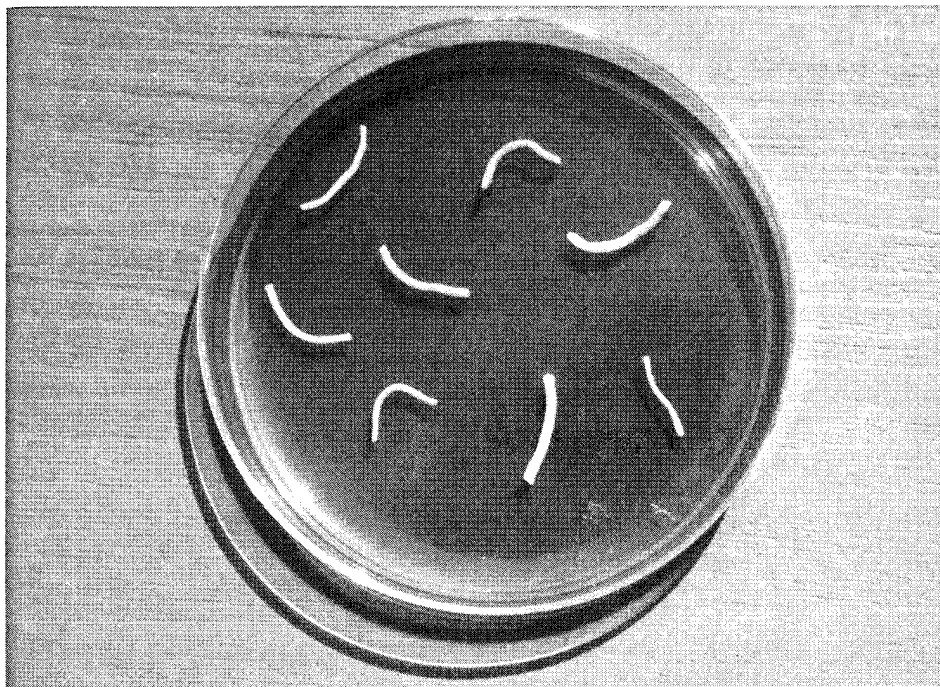


Figure 2. Aqua-Fyte string formulation used for efficacy testing on Eurasian watermilfoil growing in Murphy Hill North Pond, Guntersville, Alabama, summer 1992



Figure 3. Milfoil-infested Murphy Hill North Pond located adjacent to Guntersville Reservoir, Alabama, was used for field testing of pathogenic fungus *Mycoleptodiscus terrestris* as a biocontrol for Eurasian watermilfoil

contamination, treated and control areas were separated by 15-m buffer zones. Aboveground biomass samples were collected preinoculation and 4 weeks postinoculation from treated and untreated plots.

The mycoherbicide was applied in early July 1992 when water temperatures were within the optimum range for fungus development (20 to 28 °C). The milfoil plants had reached the water surface and had begun to form a dense canopy. The string formulation was suspended in water and applied by hand from a boat (Figure 4). Care was taken in the application to ensure even coverage over the plot surface (Figure 5).

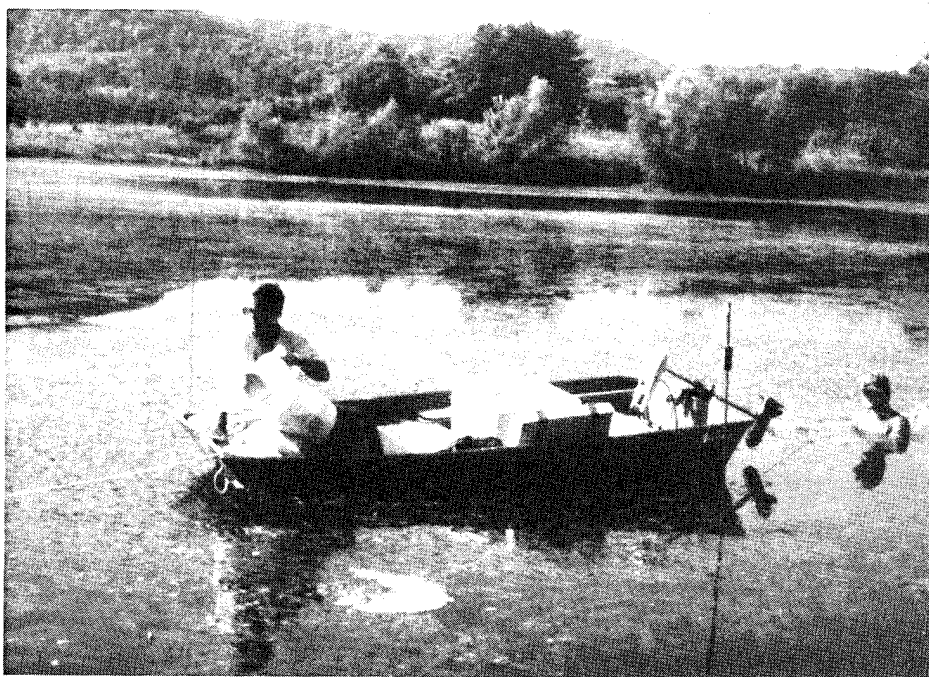


Figure 4. Hand application of mycoherbicide Aqua-Fyte at Murphy Hill North Pond, Guntersville, Alabama

One month postinoculation, biomass samples were collected at randomly assigned locations within the plots by a scuba diver using a 0.1-m<sup>2</sup> quadrat placed on the sediment surface. All rooted plant material within the quadrat was clipped at the soil surface, and all plant material was bagged and labeled. Plant samples were thoroughly washed to remove surface debris and spun dry in a washing machine set on a 6-min spin cycle. This ensured a fairly constant wet weight determination. An approximate 100-g subsample was placed in a sterile plastic bag, kept cool in an ice chest, and returned to WES for microbial analysis. Dry weight was determined by reweighing the remaining plant material and subsequently drying the sample for 5 days at 60 °C.

Total microbial counts and fungal species frequencies in milfoil plant tissue were determined by dilution plating. Ten grams of stem tissue were weighed in a sterile plastic boat, submerged in 200 ml of a 1.5-percent hypochlorite



Figure 5. Coverage of mycoherbicide Aqua-Fyte on Eurasian watermilfoil in treated plots

solution for 1 min to eliminate surface contaminants, and rinsed in tap water for 1 min. The tissue was ground with 100 ml of sterile water in a sterile blender jar for 30 sec. Aliquots of the slurry were pipetted into sterile water blanks to give dilutions of 1/100 and 1/500. After a thorough shaking, 1-ml aliquots of the dilutions were distributed over the surface of Martin's agar plates (three plates per dilution). The plates were incubated in the dark at room temperature for 5 days. Total colony counts and number of Mt colonies in each sample were determined by visual examination.

A maximum of 30 colonies from each sample were picked at random from the dilution plates and placed onto PDA slants. After a 1-week growth period at 24 °C, the slants were sorted based on various morphological characteristics of the fungal colonies including size, color, turf height and pattern, and colony reverse. Counts of morphologically similar isolates were used to determine frequencies and densities of fungal species on the field-collected milfoil.

## Mt Evaluation

Field site and sampling techniques used in the 1993 *M. terrestris* evaluation were similar to that described for the 1992 application. Plots were located in the same region of the pond but were not superimposed on plots used in the 1992 test. Plot size was reduced to 4 by 4 by 1.5 m to lessen the amount of

fungal inoculum required for the application. Treatments consisted of one application rate of *M. terrestris* broth culture paired with untreated controls.

The inoculum was prepared commercially by Ricerca, Inc., Painesville, OH. A mycelial mat was produced by growing the fungus in modified Richard's V-8 broth in two 28- $\ell$  fermentors for 72 hr. The hyphal mat was ground in a commercial blender for 30 sec to macerate the mycelium. The resulting fungal slurry was placed in sterile containers, kept cool with ice packs, and shipped directly to the field site. A small subsample of the slurry was placed in a sterile vial for determination of fungal viability, virulence, and CFU production.

Because the milfoil pathogen does not sporulate in fermentation culture, the number of CFUs in the inoculum had to be determined by dilution plating. A 1-ml aliquot of the fungal slurry was serially diluted up to a final dilution of  $1 \times 10^{-6}$ . Dilutions of  $1 \times 10^{-4}$ ,  $1 \times 10^{-5}$ , and  $1 \times 10^{-6}$  were plated onto Martin's agar in 1-ml aliquots (three plates per dilution). Plating a range of dilutions ensures that the number of CFUs in any broth culture can be determined with accuracy. When colonies were readily discernable growing on the agar surface, the number of colonies per plate were counted by macroscopic examination of the culture plates.

It was estimated that 12  $\ell$  of Mt inoculum would be needed on a per plot basis to achieve an effective application rate of 500 CFUs/milliliter. This estimate was based on the assumption that the inoculum concentration produced by industrial fermentation would be rated at  $1 \times 10^6$  CFUs/milliliter. The slurry was dispensed evenly over the surface area of the plot and allowed to naturally dissipate throughout the plant mat.

Four weeks postinoculation, plant samples from treated and untreated plots were processed as described for the 1992 application for aboveground biomass as well as microbial frequencies and densities.

### 3 Results and Discussion

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#### Pathogen Survey

Thirty-two fungal species were isolated from milfoil stem tissue collected at Mud Creek, North Sauty, and Comer Bridge sites on Guntersville Reservoir. Isolates appearing in at least two of the samples are listed in Table 1. *Mt* was found in 29 of the 30 samples, and it colonized stem pieces more frequently (71.3 percent) than any other species (Table 1). *Pythium* sp. 2, *Pythium* sp. 1, *Curvularia inaequalis*, *Cylindrocarpon destructans*, *Macrophoma* sp., and *Trichoderma* sp. were found in 60, 46.6, 26.6, 23.3, 13.3, and 10.0 percent of the samples, respectively. From among these species, only *Trichoderma* sp. would not be considered as a potential biocontrol plant pathogen.

**Table 1**  
**Frequency of Fungal Species Isolated From 30 Milfoil Samples and Diseased Stem Pieces Collected at Three Locations on Guntersville Reservoir**

Fungus	Percent Samples	Percent Stem Pieces
<i>Mycoleptodiscus terrestris</i>	96.6	71.3
<i>Pythium</i> sp. 1	46.6	04.7
<i>Pythium</i> sp. 2	60.0	04.4
<i>Curvularia inaequalis</i>	26.6	2.0
<i>Cylindrocarpon lucidum</i>	23.3	2.0
<i>Macrophoma</i> sp.	13.3	1.0
<i>Trichoderma</i> sp.	10.0	1.5
<i>Phomopsis</i> sp.	6.6	<1.0
<i>Sclerotium rolfsii</i>	6.6	<1.0
<i>Paecilomyces</i> sp.	6.6	<1.0
<i>Colletotrichum gloeosporioides</i>	6.6	<1.0
<i>Aspergillus fumigatus</i>	6.6	<1.0

That Mt was isolated with frequency from milfoil samples was unexpected. Originally described as a pathogen on soybeans (Gerdemann 1953) and reported to occur on other leguminous species (Farr et al. 1989), it was not known to commonly occur on milfoil. Since 1991, however, the fungus has been determined to occur on milfoil throughout most of its range in the United States (Figure 6).

Although present in the plant tissue at the time of the survey in 1991, *M. terrestris* did not appear to be severely impacting the milfoil populations at North Sauty, Mud Creek, and Comer Bridge. Development of a disease epidemic in plant populations is directly related to the numbers of propagative units in a pathogen population. When pathogen numbers remain low, healthy plants through natural defense mechanisms can ward off penetration and invasion of the disease-causing organisms. However, when plants are stressed, natural defense mechanisms may be rendered ineffective, and the pathogen can spread rapidly from cell to cell. Once established in plant tissues, the pathogen can reproduce resulting in a rapid buildup of more infective units. Under natural conditions in the field, Mt may only be able to incite a disease epidemic when milfoil plants are under stress from other biotic or abiotic factors. Long-term monitoring of plant and pathogen populations are necessary before an assessment of pathogen impact can be determined with accuracy.

Because accidental introductions of exotic microbes have caused disease epidemics in populations of native plant species, agencies at both the State and Federal levels have been highly conservative in granting permits for moving microbes across State lines. Finding *M. terrestris* in Alabama helped facilitate obtaining the Environmental Use Permit (EUP) to field test Mt as a biocontrol agent for milfoil. It also facilitated the issuance of a permit (PPQ 526) from the U.S. Department of Agriculture APHIS for release of the formulated fungus in Alabama.

## Mycoherbicide—Aqua-Fyte Evaluation

At 4 weeks postapplication of Aqua-Fyte, no visual differences in the plant canopy between treated and control plots were observed. Plants were topped out at the water surface and appeared green and healthy. A plant disease epidemic apparently did not develop in the mycoherbicide-treated plots. While temperatures at the surface of the plant mat reached the maximum limit for fungal infectivity (30 °C), temperatures below the surface were well within the range for optimum disease development (25 to 28 °C).

Significant differences in wet weights of aboveground biomass were not detected between treated and control plots (Figure 7). The high degree of variation between sample wet weights within plots was attributed to plant densities rather than uneven mycoherbicide application.

As in other portions of the reservoir, the Eurasian watermilfoil population at the test site has a naturally occurring population of *M. terrestris*.

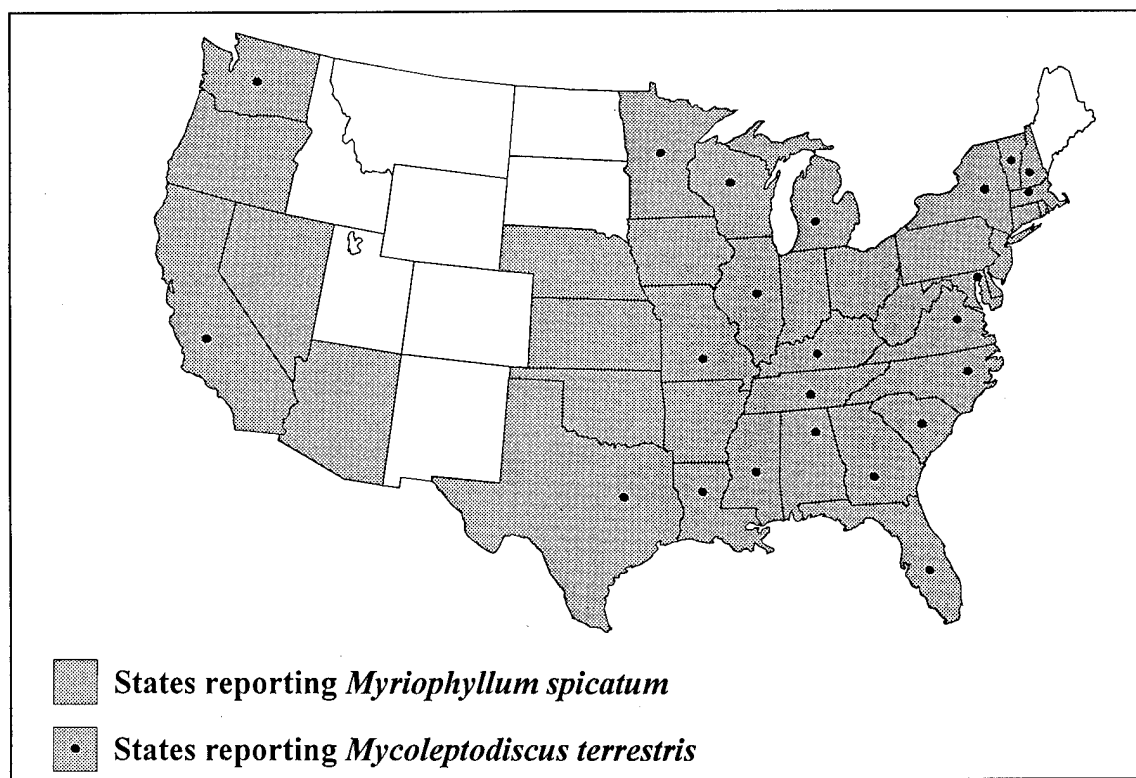


Figure 6. Distribution of *Myriophyllum spicatum* and *Mycoleptodiscus terrestris* in continental United States (Distributions were compiled from EUP submitted by EcoScience, Farr et al. (1989), personal communications, and personal collection records)

Background levels of Mt monitored prior to the study did not appear to change appreciably during the test period (Figure 8). Without a strain specific marker inserted into the active ingredient of the mycoherbicide, it was not possible to distinguish the strain of Mt used in the mycoherbicide from naturally occurring strains. It was assumed for the purposes of this study that any large increase of Mt in plant tissue following mycoherbicide application would be due to the introduced fungus.

The number of fungal CFUs in 1 g wet weight milfoil stem tissue ranged from 41.5 to 85.0 CFUs (Figure 9). Mt made up a very small proportion of the total fungal flora isolated from milfoil. A significant increase in number of CFUs of *M. terrestris* following inoculation with the mycoherbicide was not detected. The small numbers of Mt colonies isolated from stem tissue was consistent with expected background levels of endemic Mt.

Aqua-Fyte was ineffective in reducing aboveground biomass of Eurasian watermilfoil under natural conditions in the field. Poor performance of the mycoherbicide was thought to be due to fungus/formulation problems rather than biological, chemical, or physical factors encountered in the field. The problem of Mt being a weak pathogen was offset by applying the fungus at

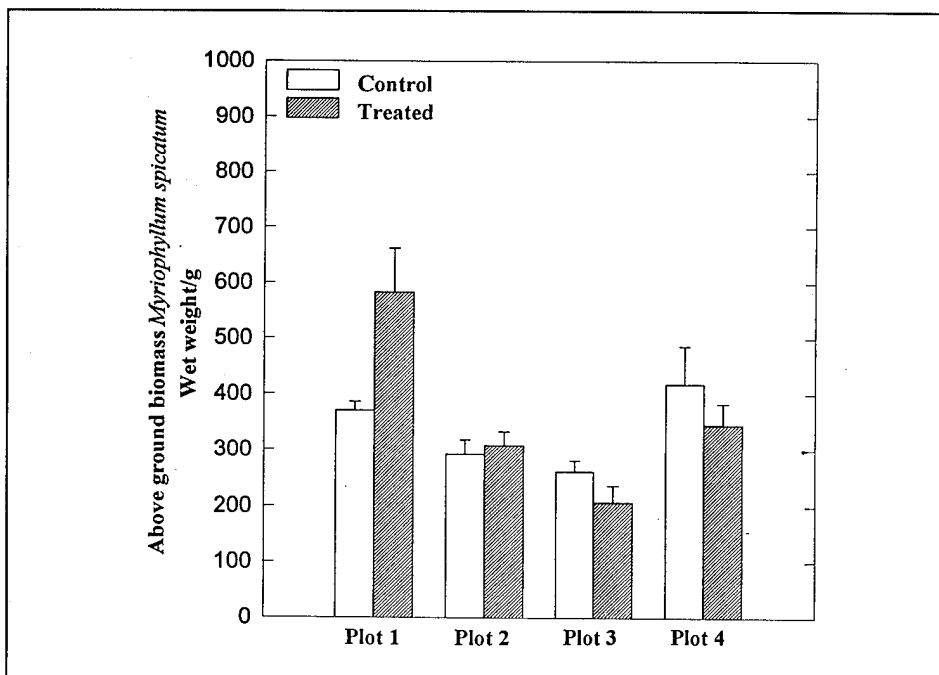


Figure 7. Eurasian watermilfoil aboveground biomass from treated and control plots 1 month postapplication with Aqua-Fyte (The values are means of wet weights SD from ten 0.1-m<sup>2</sup> quadrats)

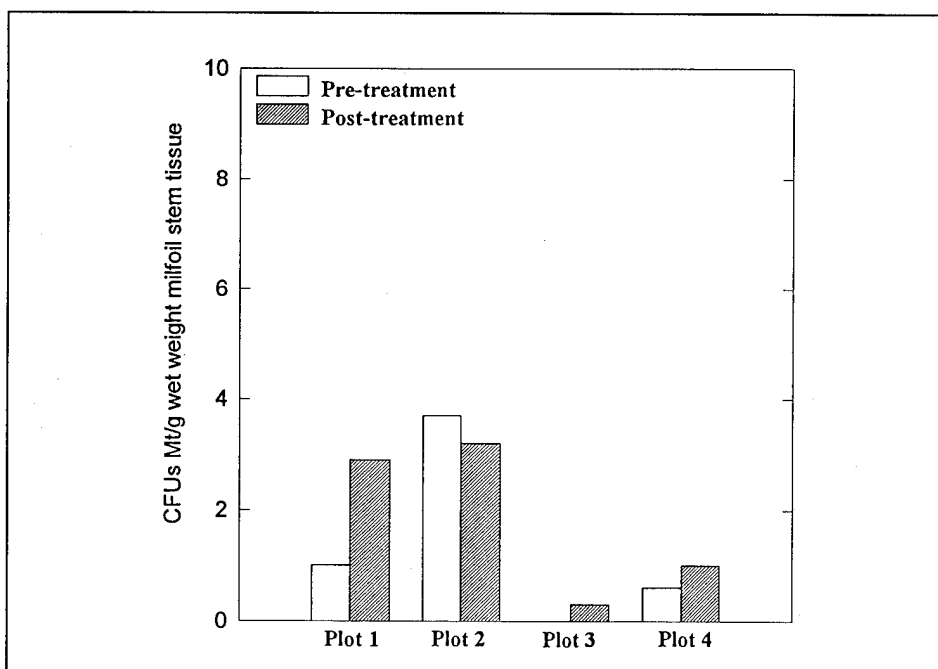


Figure 8. Number of *Mycoleptodiscus terrestris* CFUs/gram wet weight Eurasian watermilfoil stem tissue from preapplication and 1-month postapplication plant samples collected during 1992 field studies

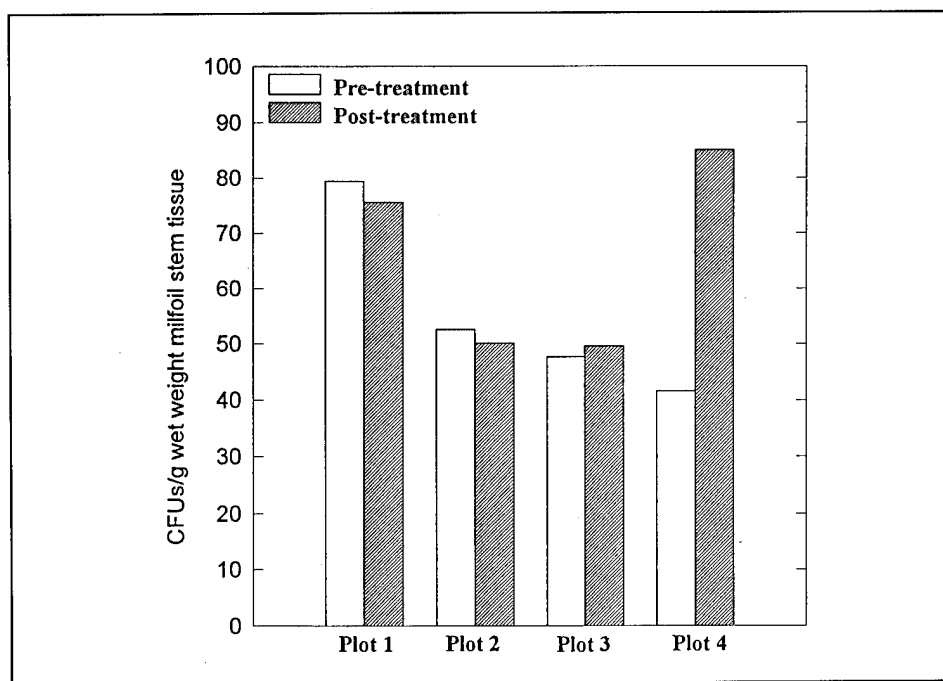


Figure 9. Fungal CFUs/gram wet weight Eurasian watermilfoil stem tissue from preapplication and 1-month postapplication plant samples collected during 1992 field studies

high dose rates in the field. Because Mt was easily reisolated from the string formulation, fungal viability was excluded as a problem.

Formulation design of the mycoherbicide appeared to lessen the impact of the fungus. Direct contact of the formulation with milfoil tissue is necessary to ensure fungal invasion. Rapid entry into the plant at multiple points is required to overwhelm plant defense mechanisms. The strings did not appear to adhere to plant surfaces at enough contact points for the fungus to become well established in plant tissues. Because the primary inoculum was ineffective, a secondary infection cycle was never initiated and a plant disease epidemic did not develop. Changes in formulation design that would allow greater coverage of the mycoherbicide over plant surfaces would ensure more rapid penetration at a greater number of contact points.

In addition, the mycoherbicide field trials indicated the need to reassess the effectiveness of the pathogen as a biocontrol agent. An application of fungal mycelium minus the inert carrier (the formulation ingredients) would provide evidence that the pathogen alone was capable of producing disease in field populations of milfoil.

## Mt Evaluation

Four weeks postapplication, significant differences in wet weights of aboveground biomass samples were not detected between treated and control plots (Figure 10). Mt inoculum concentration for the field application was determined to be  $1 \times 10^5$  CFUs/milliliter. Taking into account the immediate dilution factor of adding the inoculum to an aqueous medium, the effective CFU rate was approximately 50 CFUs/milliliter. This rate was a 10-fold reduction in the CFU counts estimated to be necessary for a successful field application.

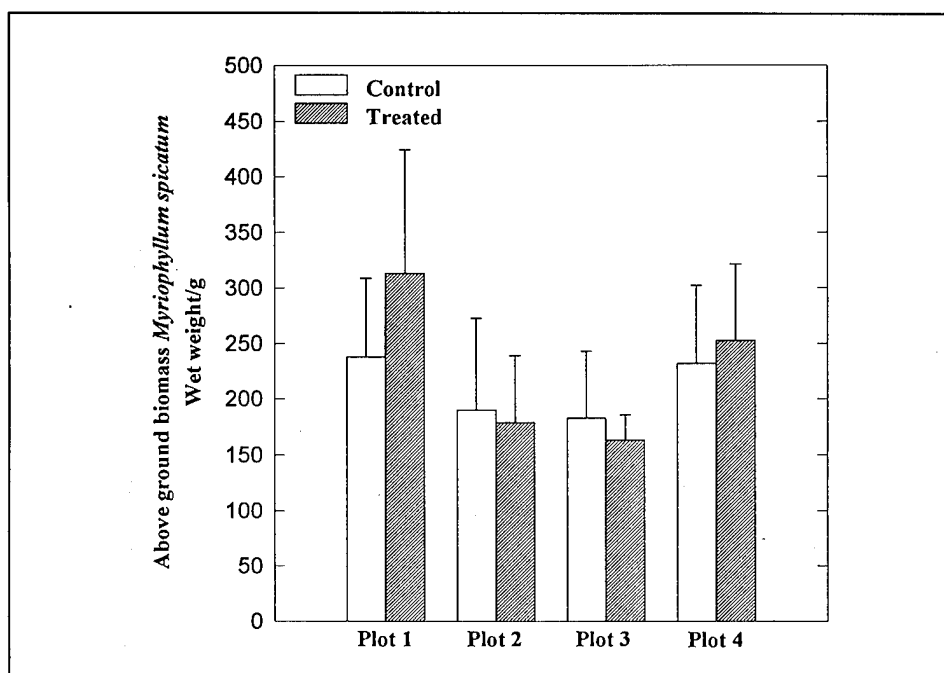


Figure 10. Eurasian watermilfoil aboveground biomass from treated and control plots 1-month postapplication with a mycelial application of *Mycoleptodiscus terrestris* (The values are means of wet weights SD from ten 0.1-m<sup>2</sup> quadrats)

In greenhouse experiments, biomass reductions have been achieved with effective CFU counts as low as 83 CFUs/milliliter. Plants are grown in containers and almost completely fill the container space. There is relatively little water movement within the column except that associated with aeration. In a field situation, plant distribution is uneven and water movements vary with environmental conditions. The fungus applied as mycelium has potentially more contact area and more contact time with stems and leaves of container-grown plants than those in the field, resulting in a greater chance for disease to develop. Inoculum applied at the same effective CFU rate (i.e., allowing for the dilution factor) would then be expected to result in a greater percent reduction of milfoil aboveground biomass in greenhouse experiments than in field experiments.

Fungus viability and virulence did not seem to be factors in the 1993 application. Mt readily grow on the selective medium used for the colony counts, thereby confirming fungus viability. The field test inoculum also reduced aboveground biomass of laboratory-grown milfoil by 80 percent compared with untreated controls.

The number of fungal CFUs in milfoil stems ranged from 40.7 to 99.6 CFUs/gram wet weight of plant tissue (Figure 11). Mt accounted for only a small percent of the total number of colonies (Figure 12). An increase in Mt was not detected between preinoculation and postinoculation samples. Had the fungus successfully invaded milfoil plants, at 4 weeks postinoculation, the numbers of Mt colonies isolated from stem tissue of treated plots would be expected to be greater than either preinoculation levels or background levels in untreated plots.

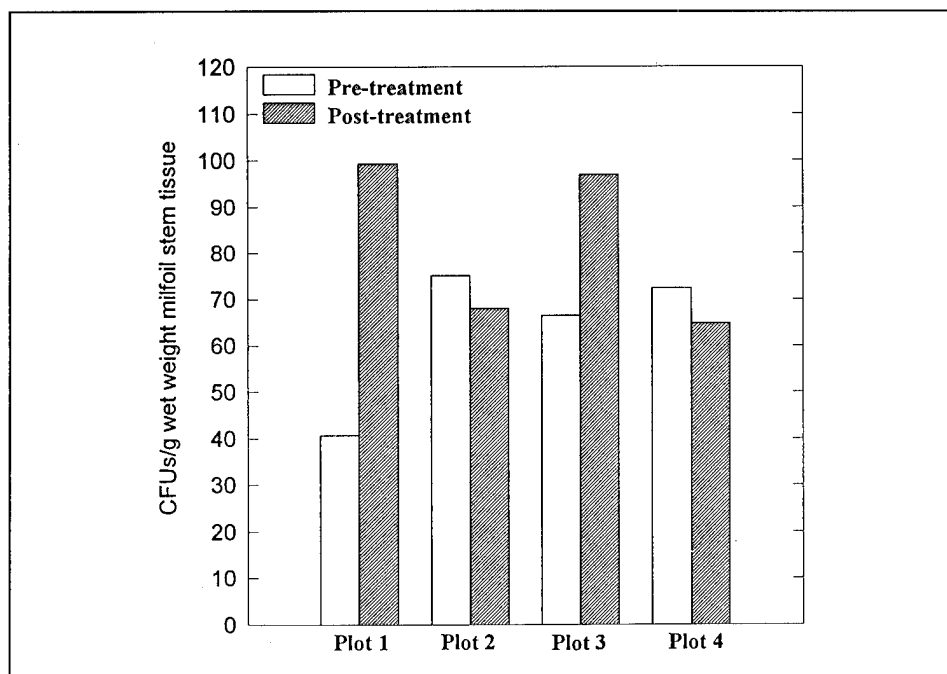


Figure 11. Number of *Mycoleptodiscus terrestris* CFUs/gram wet weight Eurasian watermilfoil stem tissue from preapplication and 1-month postapplication plant samples collected during 1993 field studies

CFUs detected in milfoil samples increased from an average of 60 CFUs/gram wet weight milfoil stem tissue in 1992 to 73 CFUs/gram in 1993. In both years, Mt made up only 5 percent of the total colony counts. Although demonstrated in the laboratory that low rates of inoculum can produce disease in milfoil, it is not known at the present time whether the endemic pathogen has or can impact established milfoil populations. A combination of three factors must be present before disease can develop: a susceptible plant, an infective pathogen, and a favorable environment (Agrios 1969). In terrestrial

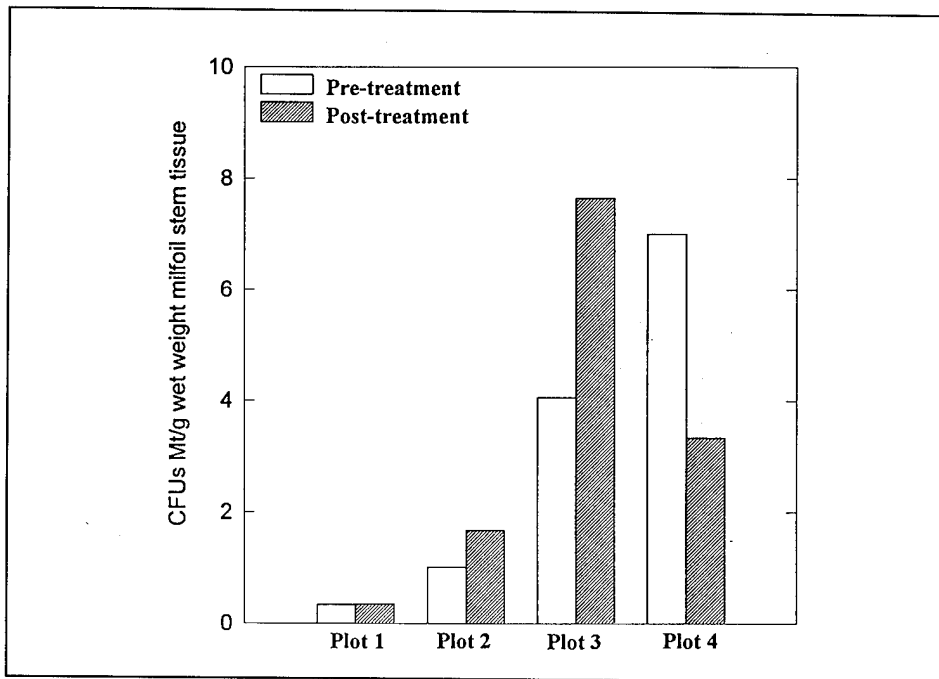


Figure 12. Fungal CFUs/gram wet weight Eurasian watermilfoil stem tissue from preapplication and 1-month postapplication plant samples collected during 1993 field studies

systems, temperature and moisture conditions are the two most critical factors that restrict or enhance disease development. Under submersed aquatic conditions, the environmental factors that are most likely to favor disease development have never been documented. Obviously moisture would not be a limiting factor. Water temperatures undoubtedly affect the growth of both plant and pathogen. Turbidity and hence light availability may be of considerable importance. A reduction in plant vigor brought about by a decrease in photosynthesis because of light availability could potentially lead to an increase in susceptibility to fungal invasion. Likewise, any changes in nutrient availability that adversely affect plant vigor could promote attack by fungal opportunists.

## 4 Conclusions

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*Mycoleptodiscus terrestris* was found to be a commonly occurring pathogen in Eurasian watermilfoil populations at three sites on Lake Guntersville Reservoir. Additional sampling in subsequent years has revealed that the fungus is widespread on milfoil populations throughout the reservoir. That isolates of the fungus are capable of causing disease on milfoil in laboratory experiments has been well documented. It is unknown, however, what conditions in the field might precipitate a disease epidemic in a milfoil population. Additional research that monitors populations of the plant and the fungus over time would help elucidate biotic conditions that could induce disease development.

The mycoherbicide Aqua-Fyte was ineffective as an alternative biocontrol resource for milfoil management. The deficiencies appear to be in the design of the formulation rather than in the biotic active ingredient. To be effective as a mycoherbicide, a formulation must have good coverage over the plant surface and have enough contact time with the plant for fungal penetration to occur. These criteria did not appear to be met with the string design of the formulation.

The pathogen *M. terrestris*, while extremely effective in greenhouse trials, has been inconsistent in its field performance. Application of the fungus as a liquid mycelial matrix has its drawbacks in that there is an immediate dilution factor and dissipation into the aqueous medium to which it is applied. Because the CFU count from the fungal fermentation used for the 1993 field test was much lower than expected (i.e., a 10-fold reduction), the requisite CFU counts for initiating disease development in the field were never achieved.

In the future, work will continue on both fungus and formulation development. Fungal enhancement can be achieved through manipulation of fermentation and milling procedures. A mass screening of all Mt isolates in the biocontrol pathogen collection will help identify virulent isolates that have additional characteristics that make them well suited for formulation incorporation (i.e., spore, chlamydospore, and/or sclerotial producers). Formulation development must focus on a carrier that will adhere to the plant at multiple points for long periods. Both efforts are necessary before the effectiveness of the fungal agent (Mt) can finally be realized.

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13. ABSTRACT (Maximum 200 words)  The use of classical biocontrol techniques for milfoil management was not a viable option when the Joint Agency Guntersville Project sponsored by the Tennessee Valley Authority and Headquarters, U.S. Army Corps of Engineers began. No host specific insects had been approved for release, and overseas searches for pathogens had not yet been initiated. A microbial biocontrol strategy, the inundative method, utilizing an endemic plant pathogen, <i>Mycoleptodiscus terrestris</i> (Gerd.) Ostazeski (Mt), known to be a parasite on milfoil, was proposed.  Introduction of microbes for biological control purposes against nuisance pest populations is strictly regulated by individual State agencies and two Federal agencies, including the U.S. Environmental Protection Agency and Agriculture Protection Health Inspection Service. The use of a pathogen that is known to be endemic within a State lessens the fear associated with introducing a microbe into a new area. Surveys were conducted on Lake Guntersville Reservoir to provide documentation on pathogens of milfoil to facilitate the permitting process from State and Federal agencies.  Thirty-two fungal species were isolated from milfoil stem tissue collected at Mud Creek, North Sauty, and Comer Bridge sites. <i>Mycoleptodiscus terrestris</i> was isolated from 29 of the 30 plant samples collected on <div style="text-align: right;">(Continued)</div>				
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Lake Guntersville. In addition to Mt, pathogenic species from the fungal genera, *Pythium*, *Curvularia*, *Cylindrocarpon*, and *Macrophoma*, were also collected.

A formulation of the endemic pathogen Mt had been developed by EcoScience Inc., Worcester, MA. The mycelium of the fungus was grown in large fermentors, incorporated into a calcium alginate matrix, and extruded as strings approximately 2 by 20 mm. The formulated fungus or mycoherbicide was registered under the trade name Aqua-Fyte. Evaluation of the mycoherbicide under field conditions was undertaken in the summer of 1992.

Aqua-Fyte was ineffective in reducing aboveground biomass of Eurasian watermilfoil under natural conditions in the field. Poor performance of the mycoherbicide was thought to be due to formulation problems rather than biological, chemical, or physical factors encountered in the field. The formulated strings did not appear to adhere to plant surfaces at enough contact points for the fungus to become well established in plant tissue and induce a disease epidemic.

Because Aqua-Fyte did not produce the desired results in the field, the fungal component of the formulation needed to be reevaluated for its effectiveness as a biocontrol agent for milfoil. The mycelium of Mt applied as an aqueous medium to milfoil in laboratory and greenhouse experiments successfully produced disease symptoms in stem and leaf tissue and reduced aboveground biomass. However, when applied to milfoil in the field, the mycelium failed to produce a disease epidemic. Applying the fungus in mycelial form in the field has its drawbacks because there is immediate dissipation and dilution when adding the fungus as a liquified matrix to an aqueous medium.

Before the inundative strategy of biocontrol can successfully be applied to management of submersed aquatic plants, research must focus on the development of carrier compounds that will adhere to the target plant at multiple points for long periods of time. Further pathogen research must address methods to enhance the fungal component through strain improvement, fermentation technology, and drying.

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